

Amendment to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (Currently amended) A method for detecting the presence or absence of at least one a first target nucleic acid sequence and a second target nucleic acid sequence in a sample comprising:

forming a ligation reaction composition comprising the sample, and a first ligation probe set for each the first target nucleic acid sequence, and a second ligation probe set for the second target nucleic acid sequence, the first ligation probe set comprising (a) at least one first probe, comprising a target-specific portion and a 5' primer-specific portion, wherein the 5' primer-specific portion comprises a sequence, and (b) at least one second probe, comprising a target-specific portion and a 3' primer-specific portion, wherein the 3' primer-specific portion comprises a sequence, and the second ligation probe set comprising (a) at least one first probe, comprising a target-specific portion and a 5' primer-specific portion, wherein the 5' primer-specific portion comprises a sequence; wherein the probes in each set are suitable for ligation together when hybridized adjacent to one another on a complementary target sequence, wherein the sequence of the 5' primer-specific portion of the first probe of the first probe set is different from the sequence of the 5' primer-specific portion of the first probe of the second probe set, wherein the sequence of the 3' primer-specific portion of the second probe of the first probe set is different from the sequence of the 3'

primer-specific portion of the second probe of the second probe, or both the sequence of the 5' primer-specific portion of the first probe of the first probe set is different from the sequence of the 5' primer-specific portion of the first probe of the second probe set and the sequence of the 3' primer-specific portion of the second probe of the first probe set is different from the sequence of the 3' primer-specific portion of the second probe of the second probe set, and wherein the first target nucleic acid sequence is different from the second target nucleic acid sequence;

forming a first test composition and a second test composition by
subjecting the ligation reaction composition to at least one cycle of ligation,
wherein adjacently hybridizing complementary probes of the first ligation probe set are ligated to one another to form a first ligation product comprising the 5' primer-specific portion, the target-specific portions, and the 3' primer-specific portion, and wherein adjacently hybridizing complementary probes of the second ligation probe set are ligated to one another to form a second ligation product comprising the 5' primer-specific portion, the target-specific portion, and the 3' primer-specific portion;

~~forming at least one~~ a first amplification reaction composition in a first amplification reaction mixture comprising: at least a portion of the first test composition; a polymerase; a double-stranded-dependent specific label, wherein the double-stranded-dependent label has a first detectable signal value when the double-stranded-dependent label is not exposed to double-stranded nucleic acid; ~~and at least one~~ a first primer set, the first primer set comprising (i) at least one a

first primer comprising the sequence of the 5' primer-specific portion of the first ligation product, and (ii) ~~at least one~~ a second primer comprising a sequence complementary to the sequence of the 3' primer-specific portion of the first ligation product;

forming a second amplification reaction composition in a second amplification reaction mixture comprising: a portion of the second test composition; a polymerase; a double-stranded-dependent specific label, wherein the double-stranded-dependent label has a first detectable signal value when the double-stranded-dependent label is not exposed to double-stranded nucleic acid; a second primer set, the second primer set comprising (i) a first primer comprising the sequence of the 5' primer-specific portion of the second ligation product, and (ii) a second primer comprising a sequence complementary to the sequence of the 3' primer-specific portion of the second ligation product;

subjecting the ~~at least one~~ first amplification reaction composition to at least one amplification reaction; and

subjecting the second amplification reaction composition to at least one amplification reaction;

detecting a second detectable signal value at least one of during and after the at least one first amplification reaction, wherein a threshold difference between the first detectable signal value and the second detectable signal value indicates the presence of the first target nucleic acid sequence, and wherein no threshold difference between the first detectable signal value and the second

detectable signal value indicates the absence of the first target nucleic acid sequence; and,

detecting a second detectable signal value at least one of during and after the at least one second amplification reaction, wherein a threshold difference between the first detectable signal value and the second detectable signal value indicates the presence of the second target nucleic acid sequence, and wherein no threshold difference between the first detectable signal value and the second detectable signal value indicates the absence of the second target nucleic acid sequence.

2 – 5 (Canceled)

6. (Currently amended) The method of ~~any one of claims 2 to 5~~ claim 1, wherein the first target nucleic acid sequence and the second target nucleic acid sequence have different nucleotides at a given locus.

7. (Currently amended) A method for detecting the presence or absence of at ~~least one~~ a first target nucleic acid sequence and a second target nucleic acid sequence in a sample comprising:

forming a ligation reaction composition comprising the sample, and a first ligation probe set for ~~each~~ the first target nucleic acid sequence, and a second ligation probe set for the second target nucleic acid sequence, the first ligation probe set comprising (a) at least one first probe, comprising a target-specific portion and a 5' primer-specific portion, wherein the 5' primer-specific portion comprises a sequence, and (b) at least one second probe, comprising a target-specific portion and a 3' primer-specific portion, wherein the 3' primer-specific

portion comprises a sequence, and the second ligation probe set comprising (a)
at least one first probe, comprising a target-specific portion and a 5' primer-
specific portion, wherein the 5' primer-specific portion comprises a sequence;
wherein the probes in each set are suitable for ligation together when hybridized
adjacent to one another on a complementary target sequence, wherein the
sequence of the 5' primer-specific portion of the first probe of the first probe set is
different from the sequence of the 5' primer-specific portion of the first probe of
the second probe set, wherein the sequence of the 3' primer-specific portion of
the second probe of the first probe set is different from the sequence of the 3'
primer-specific portion of the second probe of the second probe, or both the
sequence of the 5' primer-specific portion of the first probe of the first probe set is
different from the sequence of the 5' primer-specific portion of the first probe of
the second probe set and the sequence of the 3' primer-specific portion of the
second probe of the first probe set is different from the sequence of the 3' primer-
specific portion of the second probe of the second probe set, and wherein the
first target nucleic acid sequence is different from the second target nucleic acid
sequence;

forming a first test composition and a second test composition by
subjecting the ligation reaction composition to at least one cycle of ligation,
wherein adjacently hybridizing complementary probes of the first ligation probe
set are ligated to one another to form a first ligation product comprising the 5'
primer-specific portion, the target-specific portions, and the 3' primer-specific
portion, and wherein adjacently hybridizing complementary probes of the second

ligation probe set are ligated to one another to form a second ligation product comprising the 5' primer-specific portion, the target-specific portion, and the 3' primer-specific portion;

forming at least one a first amplification reaction composition in a first amplification reaction mixture comprising: at least a portion of the first test composition, a polymerase, a double-stranded-dependent label; and at least one a first primer set, the first primer set comprising (i) at least one a first primer comprising the sequence of the 5' primer-specific portion of the first ligation product, and (ii) at least one a second primer comprising a sequence complementary to the sequence of the 3' primer-specific portion of the first ligation product;

forming a second amplification reaction composition in a second amplification reaction mixture comprising: at least a portion of the second test composition, a polymerase, a double-stranded-dependent label; and a second primer set, the second primer set comprising (i) a first primer comprising the sequence of the 5' primer-specific portion of the second ligation product, and (ii) a second primer comprising a sequence complementary to the sequence of the 3' primer-specific portion of the second ligation product;

subjecting the at least one first amplification reaction composition to at least one first amplification reaction; and detecting the presence or absence of the first target nucleic acid sequence by monitoring a signal at least one of during and after the at least one first amplification reaction; and,

subjecting the at least one second amplification reaction composition to at least one second amplification reaction; and detecting the presence or absence of the second target nucleic acid sequence by monitoring a signal at least one of during and after the at least one second amplification reaction.

8. (Original) The method of claim 7: wherein the detecting comprises determining a threshold cycle (C_t) value from the monitoring of the signal.

9. (Original) The method of claim 7: wherein the detecting comprises determining a threshold time (T_t) value from the monitoring of the signal.

10 - 11 (Canceled)

12. (Currently amended) The method of claim 44 7, wherein the detecting of the presence or absence of the first target nucleic acid sequence comprises determining a first C_t value from the monitoring of the signal of the at least one amplification reaction of the first amplification reaction composition; and the detecting of the presence or absence of the second target nucleic acid sequence comprises determining a second C_t value from the monitoring of the signal of the at least one amplification reaction of the second amplification reaction composition.

13. (Original) The method of claim 12, wherein the detecting of the presence or absence of the first target nucleic acid sequence and the second target nucleic acid sequence comprises comparing the first C_t value to the second C_t value.

14. (Original) The method of claim 12, wherein the first target nucleic acid sequence and the second target nucleic acid sequence have different nucleolides at a given locus, and the detecting of the presence or absence of the

first target nucleic acid sequence and the second target nucleic acid sequence comprises comparing the first C_t value to the second C_t value.

15. (Currently amended) The method of claim ~~14~~ 7, wherein the detecting of the presence or absence of the first target nucleic acid sequence comprises determining a first T_t value from the monitoring of the signal of the at least one amplification reaction of the first amplification reaction composition; and the detecting of the presence or absence of the second target nucleic acid sequence comprises determining a second T_t value from the monitoring of the signal of the at least one amplification reaction of the second amplification reaction composition.

16. (Original) The method of claim 15, wherein the detecting of the presence or absence of the first target nucleic acid sequence and the second target nucleic acid sequence comprises comparing the first T_t value to the second T_t value.

17. (Original) The method of claim 15, wherein the first target nucleic acid sequence and the second target nucleic acid sequence have different nucleotides at a given locus, and the detecting of the presence or absence of the first target nucleic acid sequence and the second target nucleic acid sequence comprises comparing the first T_t value to the second T_t value.

18 – 25 (Canceled)

26. (Currently amended) The method of ~~any one of claims 10 to 12, 15, 18 to 20, and 23~~ 7, wherein the first target nucleic acid sequence and the second target nucleic acid sequence have different nucleotides at a given locus.

27 - 48 (Canceled)

49. (Withdrawn) A kit for detecting at least one target nucleic acid sequence in a sample comprising: (a) a ligation probe set for each target nucleic acid sequence, the probe set comprising (i) at least one first probe, comprising a target-specific portion, a 5' primer-specific portion, wherein the 5' primer-specific portion comprises a sequence, and (ii) at least one second probe, comprising a target-specific portion, a 3' primer-specific portion, wherein the 3' primer-specific portion comprises a sequence, wherein the probes in each set are suitable for ligation together when hybridized adjacent to one another on a complementary target nucleic acid sequence; and (b) a double-stranded-dependent label.

50. (Withdrawn) The kit of claim 49, further comprising at least one primer set comprising (i) at least one first primer comprising the sequence of the 5' primer-specific portion of the at least one first probe, and (ii) at least one second primer comprising a sequence complementary to the sequence of the 3' primer-specific portion of the at least one second probe.

51. (Canceled)

52. (Currently amended) A method for detecting the presence or absence of at least one- a first target nucleic acid sequence and a second target nucleic acid sequence in a sample comprising:

forming a ligation reaction composition comprising the sample, poly-deoxy-inosinic-deoxy-cytidylic acid, and a first ligation probe set for each the first target nucleic acid sequence, and a second ligation probe set for the second target nucleic acid sequence, the first ligation probe set comprising (a) at least one first probe, comprising a target-specific portion and a 5' primer-specific

portion, wherein the 5' primer-specific portion comprises a sequence, and (b) at least one second probe, comprising a target-specific portion and a 3' primer-specific portion, wherein the 3' primer-specific portion comprises a sequence, and the second ligation probe set comprising (a) at least one first probe, comprising a target-specific portion and a 5' primer-specific portion, wherein the 5' primer-specific portion comprises a sequence; wherein the probes in each set are suitable for ligation together when hybridized adjacent to one another on a complementary target sequence, wherein the sequence of the 5' primer-specific portion of the first probe of the first probe set is different from the sequence of the 5' primer-specific portion of the first probe of the second probe set, wherein the sequence of the 3' primer-specific portion of the second probe of the first probe set is different from the sequence of the 3' primer-specific portion of the second probe of the second probe, or both the sequence of the 5' primer-specific portion of the first probe of the first probe set is different from the sequence of the 5' primer-specific portion of the first probe of the second probe set and the sequence of the 3' primer-specific portion of the second probe of the first probe set is different from the sequence of the 3' primer-specific portion of the second probe of the second probe set, and wherein the first target nucleic acid sequence is different from the second target nucleic acid sequence;

forming a first test composition and a second test composition by subjecting the ligation reaction composition to at least one cycle of ligation, wherein adjacently hybridizing complementary probes of the first ligation probe set are ligated to one another to form a first ligation product comprising the 5'

primer-specific portion, the target-specific portions, and the 3' primer-specific portion, and wherein adjacently hybridizing complementary probes of the second ligation probe set are ligated to one another to form a second ligation product comprising the 5' primer-specific portion, the target-specific portion, and the 3' primer-specific portion;

~~forming at least one~~ a first amplification reaction composition in a first amplification reaction mixture comprising: at least a portion of the first test composition; a polymerase; and ~~at least one~~ a first primer set, the first primer set comprising (i) ~~at least one~~ a first primer comprising the sequence of the 5' primer-specific portion of the first ligation product, and (ii) ~~at least one~~ a second primer comprising a sequence complementary to the sequence of the 3' primer-specific portion of the first ligation product;

forming a second amplification reaction composition in a second amplification reaction mixture comprising: at least a portion of the second test composition; a polymerase; and a second primer set, the second primer set comprising (i) a first primer comprising the sequence of the 5' primer-specific portion of the second ligation product, and (ii) a second primer comprising a sequence complementary to the sequence of the 3' primer-specific portion of the second ligation product;

subjecting the ~~at least one~~ first amplification reaction composition to at least one first amplification reaction; and detecting the presence or absence of the first target nucleic acid sequence by detecting whether the at least one first

amplification reaction results in amplification product from the first ligation product; and,

subjecting the at least one second amplification reaction composition to at least one second amplification reaction; and detecting the presence or absence of the second target nucleic acid sequence by detecting whether the at least second amplification reaction results in amplification product from the second ligation product.

53. (Withdrawn) A kit for detecting at least one target nucleic acid sequence in a sample comprising: (a) a ligation probe set for each target nucleic acid sequence, the probe set comprising (i) at least one first probe, comprising a target-specific portion, a 5' primer-specific specific portion, wherein the 5' primer-specific portion comprises a sequence, and (ii) at least one second probe, comprising a target-specific portion, a 3' primer-specific portion, wherein the 3' primer-specific portion comprises a sequence, wherein the probes in each set are suitable for ligation together when hybridized adjacent to one another on a complementary target nucleic acid sequence; and (b) a buffer comprising poly-deoxy-inosinic-deoxy-cytidylic acid.

54. (Withdrawn) The kit of claim 53, further comprising at least one primer set comprising (i) at least one first primer comprising the sequence of the 5' primer-specific portion of the at least one first probe, and (ii) at least one second primer comprising a sequence complementary to the sequence of the 3' primer-specific portion of the at least one second probe.

55. (Withdrawn) A composition for a ligation reaction comprising a ligase and poly-deoxy-inosinic-deoxy-cytidylic acid.